THE ETIOLOGY AND EXPERIMENTAL PRODUCTION OF ERYTHEMA NODOSUM *

EDWARD C. ROSENOW

(From the Memorial Institute for Infectious Diseases, Chicago)

WITH PLATES 17 TO 22

Erythema nodosum occasionally attacks several members of the same family[1] and may occur in epidemic form.[2] Osler[3] especially has emphasized its close relation to rheumatism and endocarditis, and Brian[4] has pointed out that it not infrequently develops after tonsillitis. These are some of the indications that erythema nodosum is a specific infectious disease, but as yet no one appears to have demonstrated the same microorganism in the nodes in a series of cases, or produced the disease in animals. In this paper I wish to record briefly a series of cases in which a bacteriological study of the blood, of the probable infection atrium, and of excised nodes was made with almost uniformly positive results, lesions quite like those of erythema nodosum developing in animals on intravenous injection of the organisms isolated.

TECHNIC

The nodes were excised after thoroughly sterilizing the skin, usually with tincture of iodin. In some instances the excision was made by one linear incision after dissecting the skin from the infiltrated node; in other instances the skin and node were removed by an elliptical incision. The excised tissue was covered at once with sterile gauze and taken to the laboratory. Approximately one-half of the material removed was fixed in Zenker's solution or absolute alcohol, the rest being emulsified, after surface sterilization, in broth or salt solution in a mortar in a specially devised, sterile air chamber. In those cases in which the skin was also excised separate cultures were made of the node and the skin. Shake cultures of the emulsion were made in tall columns (10-12 cm.) of dextrose broth, ascites dextrose broth, and on blood agar and Loeffler's serum slants. One slant of each was incubated under anaerobic conditions. The tubes were incubated and examined daily for at least ten days before being discarded. The individual colonies were "fished" from the top when not too deeply situated, and obtained from the deeper layers by breaking the tube at the proper level by means of a glass cutter and red

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1. Knipe: Brit. Med. Jour., 1882, 2, p. 974; Demme: Fortschr. d. Med., 1888, 7, p. 241; Brömmer: Norsk Mag. f. Lägevidensk., 1906, 7, p. 34.
2. Eichhorst: Spec. Path. u. Therap., 1907, 3, p. 609.
3. Am. Jour. Med. Sc., 1904, 127, p. 1.
4. Deutsch. Arch. f. klin. Med., 1911, 104, p. 272.

hot glass bead at the end of a pipette. One or more tubes of ascites dextrose agar were always incubated as controls. Cultures from the supposed infection atrium were made in the same way. Blood was laked in distilled water after pipetting off most of the serum from the citrated blood. The serum and the sediment of the laked portion were planted into tall tubes of ascites dextrose agar in the usual way.

The animals while being chloroformed were held in the hands so as to prevent injury to the subcutaneous structures. Owing to the hair it was not usually possible to determine the presence of subcutaneous lesions before death. They were searched for in the deeper layers as the skin was removed. The animals were examined as soon after death as possible.

The portion of the node put aside for microscopic study was fixed in absolute alcohol, or Zenker's fluid, imbedded in paraffin, and stained with hematoxylin and eosin. Owing to the fact that the bacilli do not stain by the less penetrating stains and lose their stain readily when treated by Gram's method, the demonstration of bacilli in the tissues is difficult. Various staining methods were tried without success, but by decolorizing only partially to a pale but distinct blue in the Gram-Weigert method bacilli have been found in varying number in all the cases.

CASES

CASE 808.—A young woman in the service of Dr. Sippy at the Presbyterian Hospital. She came to the hospital complaining of general ill health with rather vague pains in legs and arms, which were never severe enough to keep her awake at night; subject to headaches and backache; constipated; no sore throat; appetite poor.

On November 26, 1913, patient noticed two small, reddened, raised, and tender nodules in palm of the hand and soon after some on the knees and on the anterior aspect of the legs. On November 30, a moderate number of tender, subcutaneous, erythematous nodes appeared chiefly over the legs and forearms. This was associated with a mild tonsillitis, definite enlargement of the cervical lymph glands, and marked tenderness in the muscles of the neck. Previous to the attack of erythema nodosum the temperature was practically normal, occasionally going to 99.6 F., at one time to 101 F. After the appearance of the nodes the temperature ranged from 99 to 102 F. for nearly two weeks and then gradually came down to normal, there being a slight rise the day after the administration of a small dose of a vaccine prepared from the organism isolated from a node and from the cervical lymph gland. The leukocytosis on November 28 was 16,000, on November 24, 13,400, hemoglobin 86 percent. Repeated examinations of the stools were negative The urine was normal except on December 4, when there was present a small amount of serum and nucleo-albumin.

On December 4, a navy-bean-sized lymph gland from the anterior margin of the left trapezius muscle and a portion of a subcutaneous, circumscribed, infiltrated, red area from the right forearm were removed under strict aseptic precautions by Dr. Lewis. Cultures were made at once. On December 7, forty very fine colonies in ascites dextrose agar developed from the subcutaneous node and fifteen from the lymph gland; also a smaller number of larger, more opaque, spherical colonies. Smears from the former show moderately gram-positive, non-acid fast, polymorphic, often beaded, and sometimes clubbed, bacilli. Smears from the larger colonies show small, short, gram-positive bacilli. Smears from the water of condensation of Loeffler's serum slants show the same bacillus in pure form. Sub-cultures on blood agar plates of the bacilli

gave small, moist, non-adherent, non-hemolysing colonies with a distinct yellowish-green color on transmitted light.

CASE 904.—A woman, 18 years of age, in Dr. Billings' service at the Presbyterian Hospital. On entrance she complained of enlarged, swollen, painful joints with deformities, crepitus and limitation of motion, with muscular contractures and muscle tenderness; and a considerable loss of weight. She had been subject to repeated attacks of tonsillitis. The present trouble had begun two and one-half years before with tenderness in the soles of the feet. Knees and ankles soon became swollen, leg muscles became sore with a gradual extension, and other joints and muscles became involved in the following order: hands, ankles, shoulders, elbows, wrists, spine. The patient was fairly well nourished, well developed; her frame small, bony; her movements difficult because of stiffness and pain in joints; the superficial lymph glands easily palpable. There were many carious teeth; one alveolar abscess opposite the second left lower molar; the pharynx red; tonsils large with deep crypts from which cheesy exudate was expressed. The thyroid was not enlarged and the heart tones were clear.

On February 6, 1913, tonsillectomy was followed by slight increase in temperature for a day or two. On February 16, a number of teeth were extracted, followed on the next day by fever from 102 to 105 F. for nearly three weeks, associated with pericarditis, pleurisy with effusion, bronchopneumonia, exacerbation of the joint sensitiveness, and successive crops of erythematous nodes of the skin chiefly over the forearms and legs, acute dilatation with acute multiple ulceration of the stomach shortly before death, February 28.

On February 18, cultures were made from the excised erythematous node, from the alveolar abscess, the blood, and the pleural fluid. On February 21, cultures from the subcutaneous node showed four colonies of a short, markedly polymorphic bacillus. The skin overlying the node showed one colony of staphylococcus and one colony of the bacillus. The blood showed B. welchii and the diphtheroid bacillus; the pleural fluid, the diphtheroid bacillus only; and the material from the alveolar abscess, the bacillus and streptococci. This bacillus was isolated also in moderate numbers after death from small, firm vegetations on the mitral valve and from the ulcer in the mucous membrane of the stomach.

Sections of the node showed hemorrhages, round cell and leukocytic infiltration, and a few gram-staining bacilli in the layers immediately beneath the cutis.

CASE 906.—A 9-year-old girl in the service of Dr. Hess in the Cook County Hospital. An attack of tonsillitis was followed in two weeks by general joint pains with definite swelling and redness only of the left elbow joint. As the joint symptoms disappeared, there developed without a chill a fever of 103 F. and a crop of red, tender, painful, circumscribed subcutaneous nodes, 0.5 to 2 cm., chiefly over the anterior aspect of the legs and forearms (Fig. 2). Patient did not now (two days later) complain of sore throat. She was well nourished and well developed. The tonsils were red and enlarged, crypts filled with cheesy exudate, small superficial ulcers on the right anterior pillar; cervical lymph glands palpable. Recovery uneventful.

Cultures were made from the tonsillar crypt, the ulcer in the throat, the blood, and from an excised node in the anterior aspect of the leg. Two days later the cultures from tonsils and ulcer, on blood agar plates, showed slightly hemolytic, and a few green-producing, streptococci with a few smaller, grayish-brown colonies which did not affect the medium and which showed bacilli similar to those isolated from the blood and node in pure culture. From the blood there developed four, and from the node ten, colonies, of a small, usually short,

sometimes clubbed, gram-positive, non-acid fast, non-motile diplobacillus. All gradations between cocci, diplococci, and distinct bacilli could be made out in smears from each of the colonies. Those nearer the top of the tube showed relatively more coccus forms, those deeper down more bacillary forms.

Rabbit 619.—Injected intravenously on March 6 with the growth from three Loeffler's blood serum slants and five days later with 5 c.c. of a twenty-four-hour anaerobic dextrose broth culture.

March 14, chloroformed and examined at once: Marked subcutaneous hemorrhage around place of injection; fading yellowish-brown edematous area over the inner aspect of thighs, the subcutaneous glands draining these areas being large and hyperemic; no other gross lesions. Blood, subcutaneous areas, and glands yielded streptococci and bacillary forms similar to those in the patient.

Rabbit 620.—Injected intravenously on March 7 with 5 c.c. of an anaerobic culture in ascites dextrose broth with sterile tissue.

March 9, seemed well; no arthritis; marked redness around place of injection.

March 10, found dead: Three subcutaneous, erythematous areas (0.5 to 1.5 cm.), one on outer aspect of right thigh, one over the anterior aspect of the chest wall, the other at the base of the left ear. The area in each instance was situated around easily visible blood vessels. The subcutaneous lymph glands draining two of these areas were large and hyperemic; no enlargement of lymph glands otherwise. There were no other gross lesions except localized, whitish streaks in the medulla, and small, embolic, white areas in the cortex, of the kidneys. Cultures from the blood gave grayish-green colonies on blood agar plates. Smears showed mostly coccoid forms, sometimes arranged in short chains, but also as distinct bacilli. Subcultures of these in tissue broth showed distinct chains of from four to twelve members, together with bacillary forms. Cultures from the nodes and glands yielded similar colonies, but the bacillary forms predominated in numbers. The subcultures of the coccoid forms in dextrose broth showed streptococci, diplococci, many single cocci, often arranged in groups.

Rabbit 623.—Injected intravenously on March 11 with 7 c.c. of a forty-eight-hour dextrose broth culture containing sterile tissue.

March 20, seemed well; chloroformed: Fading areas of hemorrhage in the skin over the back, shoulders, and legs, and enlarged lymph glands in the axilla and groin; healing subendothelial nodule in the tricuspid valve; no other lesions. Cultures negative.

Dog 72.—Injected intravenously on March 11 with the growth from 60 c.c. ascites dextrose broth. No immediate symptoms. March 14, the injection was repeated with 15 c.c. of tissue broth culture.

March 17, seemed well; chloroformed: Five distinct, circumscribed, red, erythematous, and two hemorrhagic, areas in the more superficial layers of the skin. The largest area measured 2 by 5 cm., the next largest, over the posterior portion of the back, 2 by 3 cm., and the smallest area, 0.5 by 1 cm., over the anterior aspect of the left hind leg. A large number of small, fading hemorrhagic areas in the more superficial layers of the skin. Subcutaneous lymph glands draining two of the more edematous areas large and hyperemic. The areas usually surrounded easily visible blood vessels. A hemorrhagic area in the right eye near the limbus at the point of the insertion of the internal rectus muscle. Stomach, intestines, heart, lungs, kidneys, bladder, adrenals, thyroid, pancreas, liver, testicles, muscles, and joints showed no changes. A moderate

number of small meningeal hemorrhages. Cultures from the blood, joints, and bile, sterile. Subcutaneous areas and glands yielded both non-hemolysing and hemolysing streptococci and diphtheroid bacilli.

March 31, a stab from one of the colonies showing bacilli on blood agar, into ascites dextrose agar, containing sterile tissue in the bottom, gave cocci resembling staphylococci and diplococci at the top of the tube; gram-positive, often long, straight bacilli at the bottom near the piece of tissue, where growth is very scant; and clubbed, barred bacilli, elongated diplococci, and round cocci in the middle portion.

The affinity of this organism for the skin is shown in Table 1.

Rabbit 633.—Injected intravenously on March 22 with 6 c.c. of a twenty-four-hour culture of this strain after passage through Rabbit 629 and Rabbit 619 (Table 1). Blood agar plate cultures of the bacteria injected showed small, grayish, non-hemolysing colonies only.

March 24, seemed quite well; chloroformed: A few small, brownish, discolored hemorrhagic areas in the skin of the lower part of the thighs, and four similar areas over the front legs; six hemorrhages in capsule of liver; fluid from the knee joints turbid. Cultures from the blood and joints in dextrose broth showed short, chain-producing streptococci. Blood agar plates showed a few hemolysing, and a moderate number of non-hemolysing, gray colonies of streptococci both from the blood and joint fluid.

Rabbit 634.—Injected intravenously on March 22 with 6 c.c. of tissue broth culture of the same strain as that of streptococcus isolated from the blood of Rabbit 627. Blood agar plates of the culture injected showed small, grayish-green, non-hemolysing colonies only.

March 24, chloroformed: Four subcutaneous hemorrhages and three in the intercostal muscles and fascia; joint fluids distinctly turbid. Cultures from the blood and joints in broth yielded short, chained streptococci. On blood agar plates, blood gave gray, non-hemolysing colonies only, while the joint fluid showed these, and three hemolysing colonies as well.

Rabbit 640.—Injected intravenously on March 25 with 10 c.c. of a broth culture of the strain from the blood of Rabbit 633.

March 27, found dead: No subcutaneous hemorrhages; a few small hemorrhages in the lungs, and in the more tendinous portion of the muscles; blood markedly hemolysed; joint fluid markedly turbid; no other gross lesions. Cultures from the blood and joints showed a large number of hemolysing streptococci.

Rabbit 642.—Injected intravenously on March 28 with 4 c.c. of the broth culture of the hemolytic streptococcus isolated from the blood of Rabbit 640.

March 31, found dead: Serofibrinous pericarditis, inflammation of fascia over the left thorax, and multiple suppurative arthritis; fluid from right knee joint turbid, that from the shoulder and left knee joint less turbid, while that from the intervertebral joints was clear; no hemorrhages in the skin; kidneys showed whitish, elongated areas in the medulla and hemorrhagic infarct; no other lesions. Cultures from shoulder and knee joints and from pericardium gave a large number of hemolysing streptococci, while those from the blood gave a small number.

CASE 911.—Girl, 17 years of age, in the service of Dr. Billings' at the Presbyterian Hospital. She was suffering with malaise, severe headache, chills and fever with a dry cough, substernal pain, and dyspnea on exertion. After six days, red, tender nodules appeared under the skin on the front of the leg and

the forearm, and pain developed in the muscles of the left side of the neck and shoulder. She had had an attack of severe pain in the shoulders and neck two months before. Nutrition and development were normal; teeth decayed but gums normal; previous tonsillectomy; numerous circumscribed, red, brown, and purple, tender nodes (0.5 to 4 cm.) over lower third of thighs and legs and over both forearms with some degree of symmetry. Pericarditis with effusion was found. The thyroid gland gradually enlarged and there was developed distinct tremor of the fingers when in extension. There was no distinct swelling of joints, but tenderness of the muscles of the neck and pain in moving the left shoulder. The temperature ranged between 101 and 103 F. for ten days and then dropped to normal. Blood was normal except for a leukocytosis of 13,600. The urine gave a small amount of albumin and a moderate number of leukocytes. Two blood cultures negative.

An infiltrated node over the anterior middle portion of the thigh was excised with a small piece of skin, which was removed, cultures being made separately from the node and the skin. Five colonies of a moderately polymorphic, diphtheroid bacillus in pure culture developed from the node in ascites dextrose agar; the same bacillus grew also in the water of condensation on Loeffler's blood serum slant. Cultures from the skin gave three colonies of staphylococcus albus. Subcultures from one of the colonies in dextrose agar into dextrose broth, both with and without tissue, and on Loeffler's serum and blood agar slants, all gave rather long, often barred and clubbed, gram-positive bacilli, resembling pseudo-diphtheria bacilli. Blood agar plates from tissue broth gave rather brownish-green, moist colonies of long, barred and clubbed bacilli. Stabs into tall tubes of ascites dextrose agar with a piece of sterile tissue at the bottom gave large, clubbed bacilli at the bottom, and short, thick, granular bacilli at the top.

Guinea-Pig 1183.—Injected intravenously on March 20, with 7 c.c. of an eight-day tissue broth culture.

March 21, chloroformed: Hemorrhages in deep layers of the skin over the abdomen and in the skin over both hind legs, in each instance situated along the course of easily visible blood vessels. Cultures from the blood on blood agar plates gave ten hemolysing colonies of streptococci.

Rabbit 630.—Injected intravenously on March 20 with the growth from one blood agar slant.

March 22, seemed quite well; chloroformed: Three small subcutaneous hemorrhages, one over the shoulder, the others over the hind extremities; one hemorrhage in the tendinous portion of the flexor muscles of the leg. Cultures negative.

Guinea-Pig 1189.—Injected subcutaneously and intraperitoneally on March 23 with 10 c.c. of a tissue broth culture of the organism isolated from Guinea-pig 1183.

March 24, found dead: No lesions of the skin, except rather marked infiltration at the point of injection and a mild peritonitis. Cultures from the peritoneum gave large numbers, from the blood small numbers, of grayish, non-hemolysing, and a few moderately hemolysing, colonies of streptococci. Smears from some of the non-hemolysing colonies showed mostly cocci but also a few bacillus forms, while the hemolysing colonies showed streptococci only.

Rabbit 641.—Injected intravenously on March 26 with 10 c.c. of a twenty-four-hour tissue broth culture of the strain isolated from Guinea-pig 1189. March 28, found dead: Six small erythematous areas in deeper layers of the

skin situated along visible blood vessels; multiple arthritis; no other lesions. Cultures from the blood and joints gave a moderate number of markedly hemolysing streptococci.

CASE *929*.—Girl, 18 years of age, in service of Dr. Tice and Dr. Slaymaker, Cook County Hospital. Illness had begun one week before with pain and swelling in the legs, associated with erythematous, raised, patchy, and tender eruptions of the skin over the front of the legs; ankles and knee joints painful and stiff, but not swollen; no sore throat, no chill, but malaise and fever for some days. There was no history of rheumatism. The patient was well nourished, well developed, in good general health; teeth in fair condition; tonsils slightly enlarged; skin over the tibia presented red, raised, tender, dime-sized edematous patches. One node just below the left knee was hard, shot-like, and movable under the skin. There was a slight tenderness over the ankle and knee joints but no swelling or pain on walking. Leukocytes 11,000. Urine contained trace of albumin, a few reds, but no casts. Patient was placed on large doses of sodium salicylate and sodium bicarbonate, but new nodes developed in successive crops over the skin of the legs and forearms at intervals of from seven to ten days for five weeks. Recovery.

March 19, cultures were made from an excised subcutaneous tender node on the forearm which had appeared three days before.

March 22, approximately 1,800 colonies had developed in shake cultures and growths on all the other media of a peculiar gram-staining, non-acid fast, nonmotile, small, short, polymorphic bacillus (Figs. 3 and 4).

Guinea-Pig 1184.—Injected intravenously on March 22 with 2 c.c. of a twenty-four-hour culture in ascites dextrose broth containing sterile tissue, the organism appearing as a bacillus.

March 24, seemed quite well; chloroformed: Large number of small, subcutaneous, fading hemorrhages, most numerous over the back, most of these areas showing a distinct relation to the blood vessels; one larger hemorrhage with distinct infiltration and edema over the abdomen; one large hemorrhage under the visceral pleura; no other lesions. Cultures in broth from the blood gave streptococci producing rather large, long chains, and no bacillus forms.

Guinea-Pig 1187.—Injected intravenously on March 23 with the growth from 20 c.c. of ascites dextrose broth, the organism appearing more as a non-hemolytic streptococcus.

March 24, found dead: Moderate number of small hemorrhages in the deeper layers of the skin and flat muscles of the back and thorax; two symmetrically placed hemorrhages in the fascia and muscles of the legs; no other lesions. Cultures from the blood gave moderate number of grayish, non-hemolysing colonies and some producing a narrow, hazy zone of hemolysis.

Dog 75.—Injected intravenously on March 23 with growth from 150 c.c. of ascites dextrose broth, the organism appearing as a non-hemolysing streptococcus.

Death two hours after injection with vomiting and purging: Numerous small subcutaneous and cutaneous hemorrhages; five larger hemorrhages situated over the back and shoulder, the latter symmetrical; three hemorrhages in the flat, more tendinous, portions of muscles; two situated symmetrically in the flat muscles of the scapula; mucous membrane of the stomach and intestines markedly hemorrhagic; no other lesions except two small hemorrhages in the visceral pericardium, a number of small subendothelial hemorrhages on the ventricular side of the septum, and a large hemorrhage in a leaflet of the

aortal valve. Cultures from the blood on blood agar plates gave moderate number of small, gray, non-hemolysing colonies of diplococci, while the cultures in broth gave single cocci, diplococci, and chains of from five to ten members.

Rabbit 650.—Injected intravenously on April 14 with twenty-four-hour growth, from 35 c.c. of ascites dextrose broth, the organism resembling a streptococcus.

April 15, found dead: One small subcutaneous hemorrhage (3 by 8 mm.) around a blood vessel; pericardiac sac containing a moderate amount of turbid, slightly blood-tinged fluid; joint fluids distinctly turbid; one hemorrhage in the thymus gland; no other lesions. Cultures from the blood and joints gave a large number of gray, non-adherent, non-hemolysing colonies. Those from the pericardiac fluid gave a moderate number of colonies of cocci only.

Two other rabbits and one guinea-pig injected soon after the organism was isolated, showed skin lesions, but after cultivation for three weeks the strain lost this affinity for the skin as well as its virulence. After passing it through animals, the place of localization changed at the same time as its virulence increased and as the streptococcus forms displaced the bacilli.

Rabbit 638.—Injected intravenously on March 25 with the growth of the streptococcus from Dog 75 from 15 c.c. of ascites dextrose broth.

April 20, found dead: No gross lesions anywhere except those of the gall-bladder and liver. In the wall of the gall-bladder were twelve small, circumscribed, whitish areas. Smears from the turbid, slightly bile-tinged fluid in the gall-bladder gave large numbers of streptococci. The liver was soft, grayish-yellow in color, with areas of necrosis, smears from which showed a moderate number of gram-positive diplococci with leukocytes.

The streptococcus isolated from the gall-bladder in Rabbit 638 was injected intravenously into two rabbits, two guinea-pigs, and one dog. All the animals except one guinea-pig developed cholecystitis. Two showed hemorrhages in the mucous membrane of stomach or duodenum, two arthritis, and one myositis in addition. In one rabbit, one guinea-pig, and the dog, the organism acquired hemolytic power.

CASE 962.—Girl, 7 years of age, in the service of Dr. Rothstein and Dr. Walker at the Children's Memorial Hospital. There was no history of sore throat; measles one year ago; frequent convulsive — epileptic — seizures during past six months; appetite poor; constipated; slight blepharitis and conjunctivitis; throat and tonsils hyperemic; pyorrhea. The cultures from the throat containing diphtheria bacilli (?), 1,000 units antitoxin was given; no membrane. Several hyperemic papules appeared on arms and body. Throat became redder, both tonsils large, maxillary glands enlarged and tender, and three pea-sized, subcutaneous, bluish-red, painful and tender nodules appeared on the left forearm, one on right forearm, and several on the front of both legs; short, blowing, systolic murmur heard at apex and accentuation of pulmonic second. The tonsils were removed, followed by improvement of the lesion in the skin.

On April 22, cultures were made from blood, tonsil, and node excised twenty-two hours previously and kept on ice. The node was subsiding when excised. It yielded one small colony of a short diplobacillus-like organism in pure form; the overlying skin, two colonies of staphylococcus. The blood gave colonies of a non-hemolysing, short diplobacillus, resembling the one from the node. The tonsil yielded streptococci and clubbed, short diplobacilli.

A rabbit and a guinea-pig were injected intravenously with the mixed culture from the tonsil and a rabbit was injected with the pure culture from the blood. All developed circumscribed hemorrhages and edema of the subcutaneous tissues, the rabbits also slight arthritis and endocarditis. The guinea-pig, injected subcutaneously, showed marked edema, infiltration, and necrosis of the skin at the site of injection, also a mild arthritis, symmetrical hemorrhages of the tendinous attachment of muscles about both elbows, and subcutaneous hemorrhages over anterior aspect of both legs (Fig. 11).

CASE 32.—Boy, 9 years of age, in the service of Dr. Helmholtz at the Children's Memorial Hospital. He had painful, red, shining, circumscribed swellings on the front of the legs which came on suddenly with general malaise, fever, and sore throat, following severe wetting in a rain-storm eight days previously. The nodes on the legs appeared three days after the sore throat. The patient had had sore throat during the winter months. Tonsils were swollen.

Cultures from an excised node yielded three colonies of a diphtheroid bacillus. Tonsils were removed, and the emulsion made injected into animals.

A rabbit and one of two guinea-pigs injected with emulsion of the tonsils, showed hemorrhages in the skin. The rabbit showed, in addition, a few hemorrhages in the mucous membrane of the stomach and in the fascia of the muscles of the thorax. Diphtheroid bacilli and diplococci were isolated from the lesions.

CASE 51.—Woman, single, 21, service of Dr. Ormsby at the Presbyterian Hospital. Patient had had several attacks of rheumatism and an attack of diphtheria one month previously, when she was given antitoxin. Present illness began with malaise, fever, and an intense, persistent, frontal headache, which continued for two weeks, when there appeared painful, tender, red, indurated areas under the skin of the forearm and the front of legs and thighs, associated with a dull ache and distinct swelling in both elbow joints. The nodes appeared in successive crops. The fever ranged from 100 F. in the morning to 102 F. in the afternoon for nearly two weeks. As the nodes subsided, the temperature became normal. Tonsils were enlarged, pitted, and hyperemic; teeth decayed; the nodules in the skin well-defined, usually circular, bluish-red, from 0.5 to 6 cm. in diameter; leukocytes 13,000; urine showing moderate amount of albumin and hyalin casts, and an unusual number of leukocytes. Uneventful recovery.

The description of the microscopic appearances of a node in this case will serve to illustrate the appearances observed in others: Sections of the node showed no changes in the epidermis; the corium and subcutaneous tissue were the seat of extravasated blood, serous fluid, and a moderate leukocytic infiltration. In the center of the hemorrhagic area was a small artery plugged with leukocytes. Surrounding the vessel, and throughout the hemorrhagic area, were collections of blood pigment, which were easily seen with the low power and which were good places to search for bacteria, because it is here that they are most numerous (Fig. 3). One area in the subcutaneous tissue, just beneath the sweat glands, which showed no changes, presented dense leukocytic and round cell infiltration. Here only a few bacilli could be found, mostly within leukocytes. In all of the sections, areas of dense leukocytic infiltration were found usually situated near, or surrounding, a fair-sized artery. Thrombosis of the arteries in the center of or near areas of dense leukocytic infiltration was found in four of the cases; here bacilli were found in leukocytes within the lumen and in one within the vessel wall. The lymph channels were often dilated and filled with leukocytes (Fig. 5). The sequence of events appeared to be dilatation

and thrombosis of blood vessels and lymph channels, hemorrhage, polynuclear leukocytic infiltration, followed by invasion of plasma cells and endothelial leukocytes, the latter commonly mitotic. In no instance was there any evidence of invasion of the infection from epidermis or sweat glands. In only one case was there leukocytic infiltration around sweat glands, which were at the periphery of a large area in the subcutaneous tissue. The morphology of the organism found in the tissues corresponded quite accurately to that of those isolated in the cultures (Figs. 4, 5, 6, 17).

Owing partially at least to the fact that in the animals the tissue was removed sooner after the appearance of the lesions than in the patients, the hemorrhage was the marked feature and leukocytic infiltration relatively slight in the sections from the animals.

Here the hemorrhages and infiltration were nearly always in the deep layer of the corium, or loose subcutaneous tissue (Fig. 13). The hemorrhages usually surrounded a relatively large artery. In some of the sections the artery was the seat of thrombosis, or accumulation of leukocytes along the intima in the area of hemorrhages (Fig. 16). Some of the leukocytes contained organisms, and, in one instance, typical organisms were found directly in the wall of an artery which showed mural implantation of leukocytes (Fig. 17) adjacent to an area of hemorrhage in which exactly similar organisms were found in small numbers. Bacteria were never present in enormous numbers in the experimental lesions in the animals which were injected with the strains as isolated, and the amount of edema and leukocytic infiltration were correspondingly less than in the human lesions. However, after a number of animal passages, the lesions produced often showed the presence of many diplococci and chains (Fig. 14).

SUMMARY OF THE RESULTS AND GENERAL DISCUSSION

A diphtheroid, gram-staining, polymorphic, non-motile, non-spore-forming bacillus producing small, round colonies in dextrose agar, and small, gray, or yellowish, non-hemolysing colonies on blood agar, and having a wide range of fermentative power, was isolated from erythematous nodes removed in each of eight cases (Fig. 6). The number of colonies obtained ranged from 1 to 18,000. The nodes which were excised soon after their appearance, contained the largest number of bacteria, whereas those which had existed for from four to six days and where the symptoms were subsiding, contained fewer bacteria. A large proportion of the organisms were probably killed when the tissue was held in the Bunsen flame in sterilizing the surface, or perished

otherwise in the cultures, because the number of bacteria found in sections indicate a larger number present than is indicated by the number of colonies obtained. Thus in two sections which were examined of the node from Case 929, fully twice as many organisms were found as were indicated by the number of colonies which developed. One, two, and five colonies of staphylococcus albus were found in the node in three cases, and two colonies of B. welchii in one. In the rest, the organism appeared in pure form. The overlying skin, from which separate cultures were made in four cases, showed only three colonies of the diplobacillus and usually contained a few colonies of staphylococcus albus. The latter may be considered as of no etiologic importance, because it had no virulence and failed to produce skin lesions. The same organism as that obtained from the nodes was isolated at the same time in pure culture from the blood in two cases, and in conjunction with B. welchii in one case. The infection atrium would appear to have been the tonsils so far as the clinical history in two cases indicates; in three others the tonsils contained an organism which produced hemorrhages in the skin in animals; in another case the organism was found in a superficial ulcer in the throat, and in still another in an abscess of a tooth. In three cases the infection atrium was not apparent nor could it be determined. Constipation was present in these three cases. Throat infection was not severe at the time nodes developed, which usually was three to fourteen days after the throat symptoms were at their height. In four of the cases there was a definite but mild arthritis; in three, myositis or fibrositis; in two endocarditis; in four lymphadenitis; in two pericarditis; and in three, mild nephritis. All of the typical cases ended in recovery. The patient in whom the skin lesions were not typical and who suffered from a general invasion with the organism, died. Three cases occurred in children under 10 years of age, two girls and one boy. The other cases occurred in young women from 15 to 21 years of age. In two of the patients cultures from the throat showed bacilli indistinguishable from diphtheria bacilli; diphtheria antitoxin was given 3, and 28, days previously to the appearance of the nodes. The bacilli isolated from the nodes in these two cases were more like diphtheria bacilli than in the other cases and this circumstance suggests the possibility that the diphtheria bacillus may become so modified as to acquire affinity for the subcutaneous tissue.

In Table 1 is given a summary of the results in the animals which were injected intravenously with Strain 906. The figures in the column marked "Dose" indicate the growth from that many cubic centimeters of broth. The figure to the right and above the number of the strain injected, indicates the number of animal passages. It is seen that the organism soon after isolation has a marked affinity for the skin, which it loses on longer artificial cultivation as well as on animal passage. This fact was observed in the other cases as well and

TABLE 1

LESIONS PRODUCED BY INTRAVENOUS INJECTION OF STRAIN ISOLATED FROM CASE 906

Animal No.	Date Injected	Bacteria	Dose	Autopsy	Lesions in			
					Appendix	Stomach and Duodenum		Gall Bladder
						Hemorrhage	Ulcer	
Rabbit 619	March 6, 7, 14	906 from node	30 c.c. 5 c.c. 5 c.c.	March 14	0	0	0	0
Rabbit 620	March 7.....	906 from node	5 c.c.	March 10	0	0	0	0
Rabbit 623	March 11.....	906 from node	7 c.c.	March 20	0	0	0	0
Dog 72....	March 11-14..	906 from node	60 c.c. 15 c.c.	March 17	0	0	0	0
Rabbit 624	March 12.....	906^{2} from node	7 c.c.	March 19	0	0	0	0
Rabbit 627	March 14.....	906 from node	45 c.c.	March 20	0	0	0	0
Rabbit 629	March 16.....	906^{2} from blood of Rabbit 619	7 c.c.	March 18	0	0	0	0
Rabbit 633	March 22.....	906^{3} from blood of Rabbit 629	6 c.c.	March 24	0	0	0	0
Rabbit 634	March 22.....	906^{2} from blood of Rabbit 627	6 c.c.	March 24	0	0	0	0
Rabbit 639	March 26.....	906^{3} from blood of Rabbit 634	10 c.c.	March 28	0	0	0	0
Rabbit 640	March 26.....	906^{4} from blood of Rabbit 633	10 c.c.	March 27	0	0	0	0
Rabbit 642	March 28.....	906^{5} from blood of Rabbit 640	4 c.c.	March 31	0	0	0	0
Rabbit 645	April 6.....	906 from node..	30 c.c.	April 8	0	0	0	0
Rabbit 636	March 22.....	906 from node..	5 c.c.	March 28	0	0	0	0

found to hold with respect to the guinea-pig, rabbit, and dog. In Table 2 is given a summary of the results obtained with six strains injected intravenously when isolated and later. "When isolated" means soon after isolation, usually in the second or third culture. The tendency of the organism to localize in the subcutaneous tissue was seen in 18 of 20 animals injected with organisms soon after isolation, but in only 2 of 9 animals after cultivation for a longer time. The same strains after one to five animal passages, produced lesions in the skin in only 6 of 14 animals. These six animals were injected with strains

after only one or two animal passages, and the lesions were smaller and less numerous than those following injection of the strain when isolated. The shifting of the localization is well shown also in Table 1 in the case of Strain 906. This is not any accidental occurrence, because it was observed with all the strains and in different species. That it is due to acquired properties is illustrated in the results after injection of Strain 929,[3] which was isolated from the bile in a rabbit with cholecystitis following injection of strain 929[2] and which pro-

TABLE 1—*Continued*

LESIONS PRODUCED BY INTRAVENOUS INJECTION OF STRAIN ISOLATED FROM CASE 906

Lesions in									Remarks
Pancreas	Joints	Endocardium	Myocardium	Muscles or Fascia	Kidney	Intestines	Lung	Skin	
0	0	0	0	0	0	0	0	+++	Regional lymph glands enlarged
0	0	0	0	0	+	0	0	++	Regional lymph glands enlarged
0	0	..	0	0	0	0	0	++	Regional lymph glands enlarged
0	0	0	0	0	0	0	0	++	Subcutaneous lymph glands enrarged
0	0	0	0	+	0	0	..	+	
0	0	0	0	0	0	0	0	+	
0	0	+	+	+	0	0	..	+	Lymph gland enlarged; hemorrhages in thymus and thyroid glands
0	+	+	0	+	0	0	0	+	Hemorrhages in liver
0	+	0	0	+	0	0	0	+	Hemorrhages in thyroid gland
0	++	0	0	+	0	0	0	0	
0	+++	0	0	0	0	0	..	0	Hemolytic streptococcus from blood and joints
0	+++	0	0	0	0	0	0	0	Pericarditis
0	0	0	0	0	0	0	0	0	
0	0	0	0	0	0	0	0	0	

duced cholecystitis in both of two rabbits, one dog, and in one of two guinea-pigs.

The four instances of arthritis and endocarditis following injection of strains when isolated were mild, while the arthritis which developed after animal passage was more marked. The renal lesions consisted of infarcts, chiefly in the medullary portion (Fig. 12), and subcapsular hemorrhages. The hemorrhages observed in the stomach or duodenum followed injection of the strain isolated from the acute ulcers in the fatal case (904), or after animal passage in the other strains.

TABLE 2

LESIONS PRODUCED BY INTRAVENOUS INJECTION OF ORGANISM FROM ERYTHEMA NODOSUM WHEN ISOLATED AND AFTER ANIMAL PASSAGES AND ARTIFICIAL CULTIVATION

Condition of the Organisms	Number of Animals	Lesions in													
		Appendix	Stomach and Duodenum		Gall Bladder	Pancreas	Joints	Endocardium	Myocardium	Muscles or Fascia	Kidney	Intestines	Lung	Skin	Pericardium
			Hemorrhage	Ulcer											
When isolated	20	0	2	0	0	0	4	4	0	7	2	1	1	18	2
After one to five animal passages	14	0	3	0	7	0	7	2	2	7	1	1	6	6	1
After cultivation on artificial media for some time	9	0	2	0	1	0	1	1	0	0	0	0	0	2	0

The lesions in the subcutaneous tissue usually surrounded fair-sized blood vessels and were often symmetrically placed, especially in the extremities (Figs. 8, 9, 10, 11). They consisted first of circumscribed, rather large hemorrhages, which usually subsided without marked infiltration and edema, but in some instances there were redness of the skin and marked infiltration and edema as the hemorrhages faded. In some instances there were numerous small cutaneous lesions instead of the larger subcutaneous hemorrhage, resembling the hemorrhages in cases of "erythema multiforme." This result supports the view, held by some, that erythema multiforme is due to the same or similar cause as erythema nodosum. Subcutaneous injection in guinea-pigs of the strains as isolated, was followed by marked infiltration and necrosis of the skin at the point of injection. Intraperitoneal injection produced only slight lesions, the organisms being taken up promptly by leukocytes, but after animal passage, they produced serofibrinous peritonitis, the exudate showing little phagocytosis. Enlargement of the regional lymph glands occurred quite constantly in the animals in which lesions of the skin developed, and it made no difference whether the injection was made intravenously or subcutaneously. Cultures from the large hyperemic glands yielded the organism injected, as did the subcutaneous lesions (Figs. 14 and 15). The characteristic localization occurred after intravenous injection no matter whether the strain was isolated from the blood, the node, or the supposed infection atrium.

Since the organism resembles morphologically diphtheroid bacilli from other sources, a number of strains from Hodgkin's disease were injected as controls. None produced subcutaneous hemorrhages when injected intravenously, or infiltration when injected subcutaneously Hemorrhages under the skin occur only rarely on intravenous injection of streptococci. An organism isolated from the thyroid gland in goiter resembles somewhat the one from erythema nodosum and hemorrhages under the skin have been observed in two rabbits following intravenous injection with the thyroid strain.

In three cases it was quite impossible to decide whether the organism isolated should be regarded as a streptococcus with marked involution forms, or as a diphtheroid bacillus with streptococcal forms. In the other cases the organisms when isolated seemed to belong to the diphtheroid group. In no instance was there a mixed infection with a bacillus and a streptococcus. On cultivation in some of the media and

on injection into animals, streptococcal forms were produced freely. The results obtained from a closer study of the character of two of the strains serve to illustrate the polymorphic character of the organism.

STRAIN 906.—Isolated from a cutaneous node and the blood. Subcultures on agar plates from four colonies gave small, moist, brownish-gray, non-adherent, non-hemolysing colonies with a distinct yellowish-green color on transmitted light. Smears showed many more short bacillary forms than in the original cultures, but there were all gradations between cocci and bacilli in the colonies examined. Subcultures on blood agar slants from two of the colonies were inoculated into ascites dextrose broth in tall tubes containing sterile kidney tissue at the bottom, and into dextrose broth. In both there developed diffuse, somewhat granular turbidity in twenty-four hours, smears showing organisms often in clumps similar to those in the dextrose agar except that bacillary forms were longer and narrower, and that distinct chains of cocci could be made out. Blood agar plates from these showed again the colonies first described, but smears from some of them showed bacilli only; others bacilli, elongated diplococci, and coccus forms; while still others showed coccus forms not only appearing in clumps, indistinguishable morphologically from staphylococci, but also in short chains of from five to ten members. Subcultures of the latter yielded cocci only, some of which were in chains, while those containing bacilli yielded bacillary forms only in ascites dextrose broth.

STRAIN 929.—Isolated in pure culture from a cutaneous node. Spherical bodies were present usually at one end of the bacillary form, but were found also in the center; there was also a rather large number of free coccus forms. The coccus forms retained Gram's stain more tenaciously than the rods and at times made the bacilli appear to be club-shaped and spore-bearing. Smears from the colonies near the top of the dextrose agar cultures showed the bacillary forms to be longer and narrower than those from the bottom of the tubes. All gradations in form between straight and clubbed bacilli, some of which retained Gram's stain while others did not, and gram-positive, elongated diplococci, and perfectly round coccus forms, were found in each of the ten colonies examined. Anaerobic cultures on Loeffler's serum which showed bacilli the day before and were incubated under aerobic conditions over night, presented most marked involution forms — numerous small cocci, and larger oval or round bodies both free, and in the middle, and at the end, of bacilli. The aerobic cultures on Loeffler's serum and blood agar showed a predominance of diplococcal forms often in short chains instead of bacilli. From one of the original colonies in the ascites dextrose agar there were inoculated ascites dextrose broth, with and without sterile tissue. and ascites dextrose agar in stab. The former yielded short, chain-forming cocci, while the broth containing the tissue yielded the bacillary forms only and the stab in ascites-dextrose-agar both varieties. Blood agar plates from ascites dextrose broth without tissue showed small, grayish, non-hemolysing, non-adherent colonies of small diplococci forming short chains, while the culture containing the tissue showed somewhat larger, brownish-yellow, more elevated, more opaque, non-hemolysing colonies containing bacillary forms only, which produce distinct foul odor.

Cultures were made from the blood in ten animals and from the subcutaneous lesions and enlarged adjacent lymph glands in seven animals, after intravenous injection with the organism in bacillary form. In six the blood yielded a pure culture of non-hemolysing coccoid forms in chains, in two bacillary and

streptococcal forms, and in two bacillary forms only; while the cultures from the subcutaneous node and lymph gland showed bacillary forms only in four, and both bacillary and streptococcal form in the other three. The strains isolated as bacilli after one animal passage assumed streptococcal form in the second animal passage (Figs. 6 and 7). That the streptococcal forms isolated from the animals really were changed bacillary forms and not accidental invaders from another source seems certain, because exactly similar streptococcal forms were isolated simultaneously in some instances from dog, rabbit, and guinea-pig.

Three of the strains of streptococcal form acquired hemolytic properties on animal passage. Strain 906 appearing as a non-hemolysing streptococcal organism after three animal passages, was injected into two rabbits, two guinea-pigs, and one dog. The blood or joint exudate from all yielded hemolysing streptococcal forms immediately after death from chloroform.

CONCLUSIONS

As a result of this study it appears that erythema nodosum is due to a diphtheroid bacillus, closely resembling in some stages the streptococcus group. This organism has an elective affinity for the subcutaneous tissues. The infection atrium and the place where the organism acquires the affinity for the skin appears commonly to be in the tonsils and pus pockets about the teeth. The reason for the pain in the cutaneous node is probably due to the fact that the hemorrhage, infiltration, and edema surround a relatively large blood vessel and hence the adjoining nerve trunk.

EXPLANATION OF PLATES 17 TO 22

PLATE 17

Fig. 1.—Cutaneous node from erythema nodosum in man (Case 51). Section showing infiltration of the subcutaneous tissue surrounding a thrombosed artery. Note the complete absence of involvement of cutis and only slight infiltration of the deeper layers of corium. × 17.

Fig. 2.—Subcutaneous tissue from erythema nodosum in man (Case 906). Section showing marked leukocytic and round cell infiltration along the connective tissue strands between the layers of fat. × 53.

PLATE 18

Fig. 3.—Subcutaneous tissue from erythema nodosum in man (Case 929). Section showing marked hemorrhage and beginning leukocytic infiltration and deposits of blood pigment.

Fig. 4.—Subcutaneous tissue from erythema nodosum in man (Case 929). Section showing red blood corpuscles, blood pigment, nuclei of disintegrated leukocytes and diplococci and diphtheroid bacilli.

Fig. 5.—Thrombosed artery in Fig. 1 showing a diphtheroid bacillus in the thrombus.

Fig. 6.—Smear from a single colony in ascites dextrose agar 72 hours after inoculation with the emulsion of the subcutaneous node in Case 929, showing diphtheroid bacilli.

Fig. 7.—Smear from blood of guinea pig injected with Strain 929, after one animal passage, showing typical diplococci in chains.

PLATE 19

Fig. 8.—Experimental erythema nodosum in rabbit. Skin showing an area of circumscribed subcutaneous hemorrhage and infiltration and an enlarged lymph gland, 72 hours after intravenous injection of the diphtheroid bacillus when isolated from the node in erythema nodosum in man (Case 906).

Fig. 9.—Experimental erythema nodosum in dog. Skin showing an area of circumscribed subcutaneous hemorrhage and infiltration, 72 hours after intravenous injection of the diphtheriod bacillus from the node in erythema nodosum in man (Case 906).

PLATE 20

Fig. 10.—Photograph showing circumscribed hemorrhages of the skin and symmetrical hemorrhages of the fascia of the interior aspect of the tibiae in rabbit, 48 hours after an intravenous injection of a diphtheroid bacillus from the subcutaneous node in erythema nodosum in man (Case 904).

Fig. 11.—Photograph showing symmetrical hemorrhages in the subcutaneous fascia of the elbows of guinea pig, 48 hours after intravenous injection of the diplobacillus from the blood in erythema nodosum in man (Case 962).

PLATE 21

Fig. 12.—Photograph of hemorrhagic infarct in the kidney and localized fibrositis and myositis over the thorax in a guinea pig, 72 hours after an intravenous injection of the strain from erythema nodosum after four animal passages.

Fig. 13.—Skin of rabbit. Section showing hemorrhage, and leukocytic and round cell infiltration of the subcutaneous tissue spaces, 72 hours after intravenous injection of the diphtheroid bacillus, see Fig. 8. Note the complete absence of involvement of the cutis and only slight infiltration in the corium.

PLATE 22

Fig. 14.—A diplococcus in the area of infiltration shown in Fig. 13.

Fig. 15.—Diplococci in a hemorrhagic lymph gland 48 hours after an intravenous injection of the diphtheroid bacillus after two animal passages.

Fig. 16.—Section of the artery from the area of subcutaneous hemorrhage shown in Fig. 9, showing mural aggregation of leukocytes.

Fig. 17.—Diplobacilli in the wall of the artery shown in Fig. 16.

PLATE 17

Fig. 1

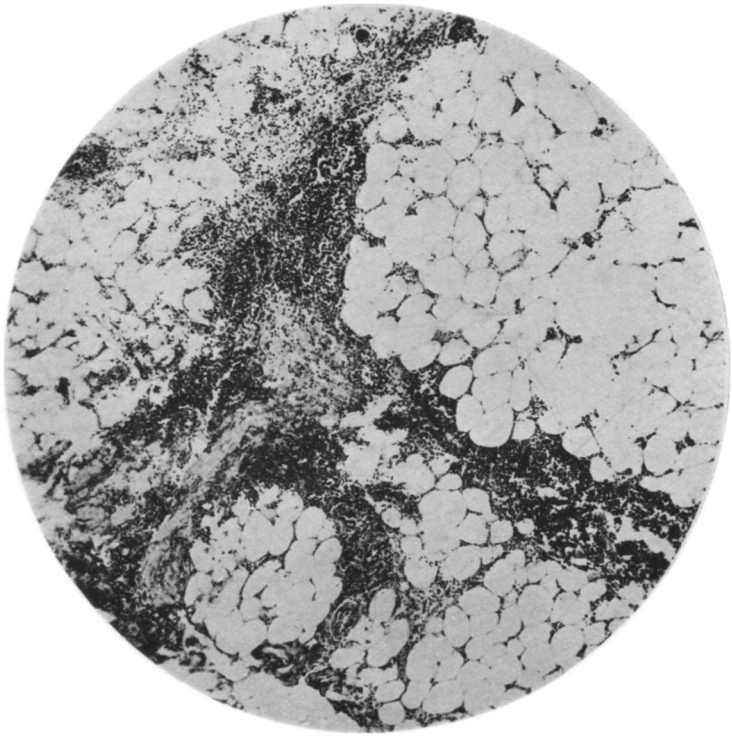

Fig. 2

PLATE 18

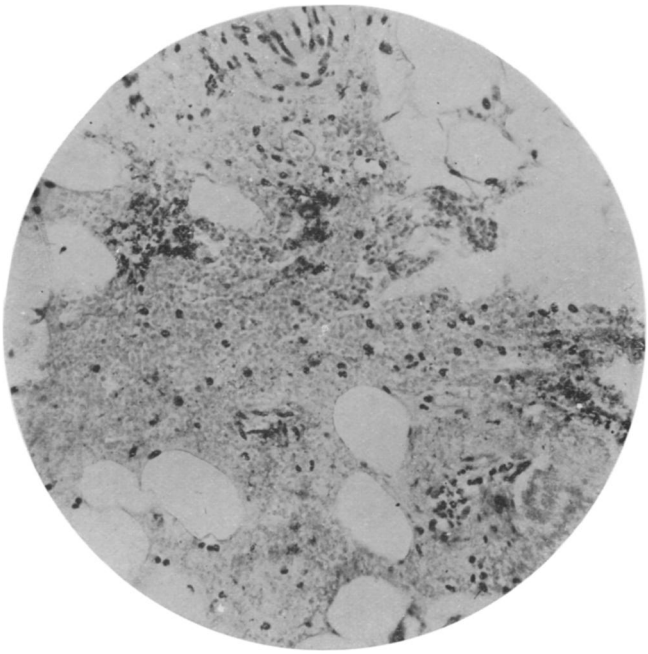

Fig. 3

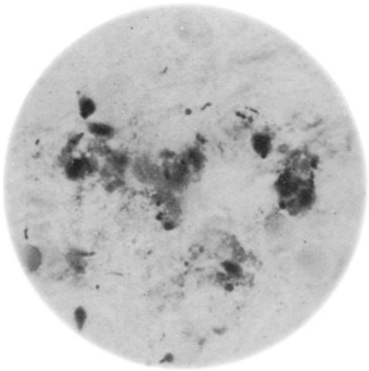

Fig. 4

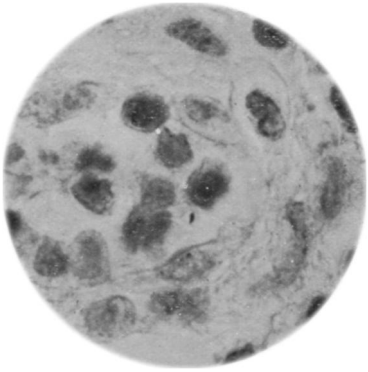

Fig. 5

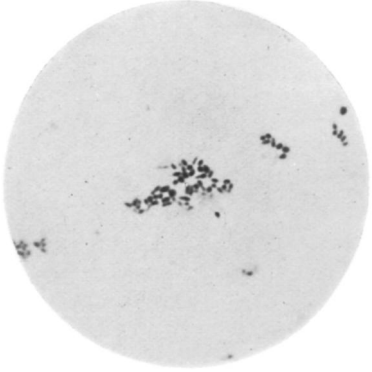

Fig. 6

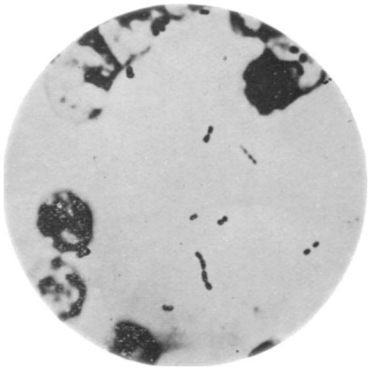

Fig. 7

PLATE 19

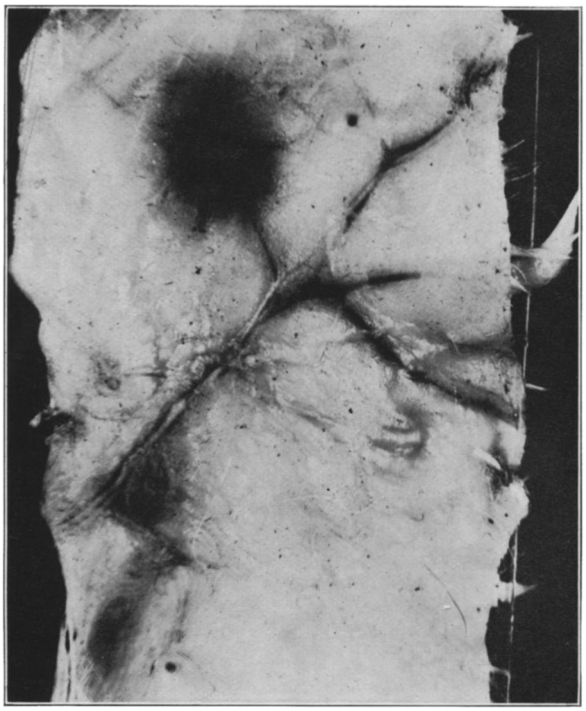

Fig. 8

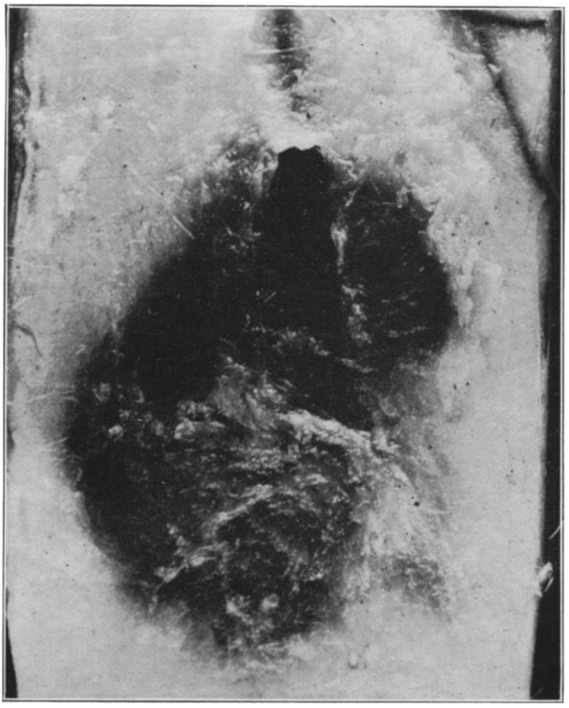

Fig. 9

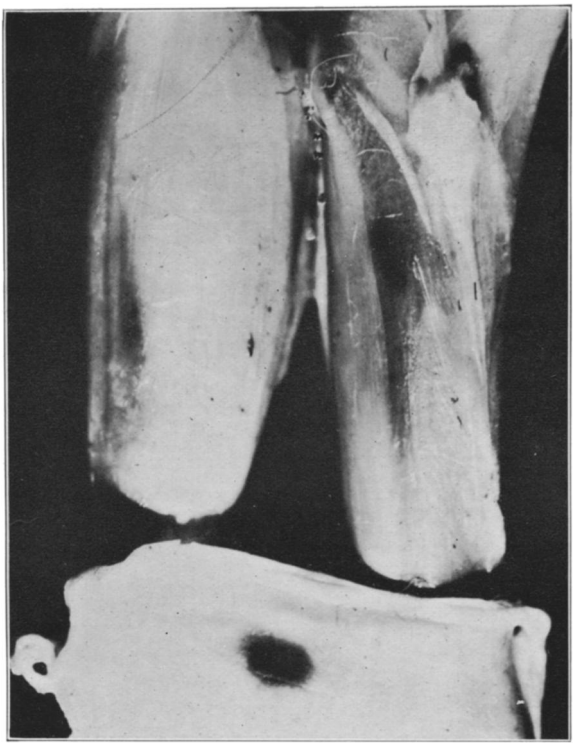

Fig. 10

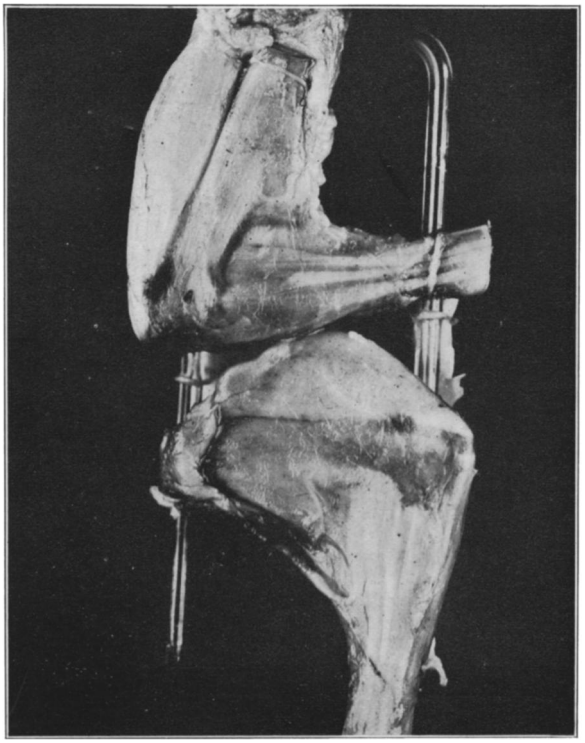

Fig. 11

PLATE 21

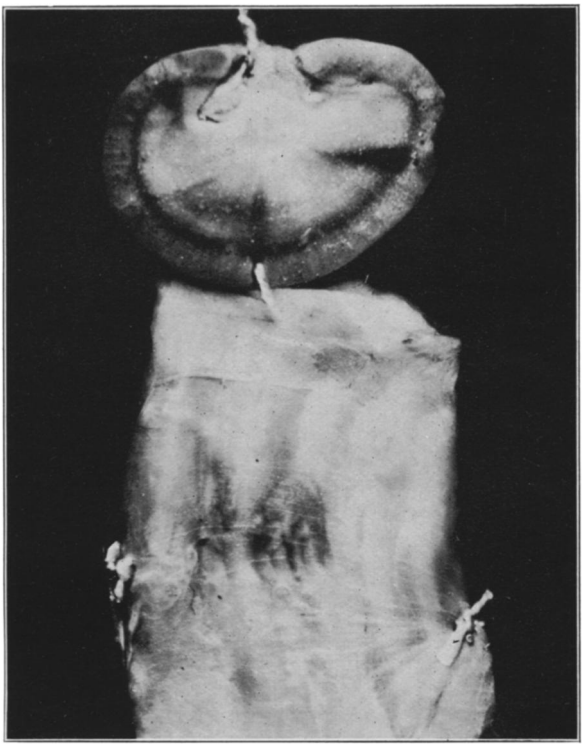

Fig. 12

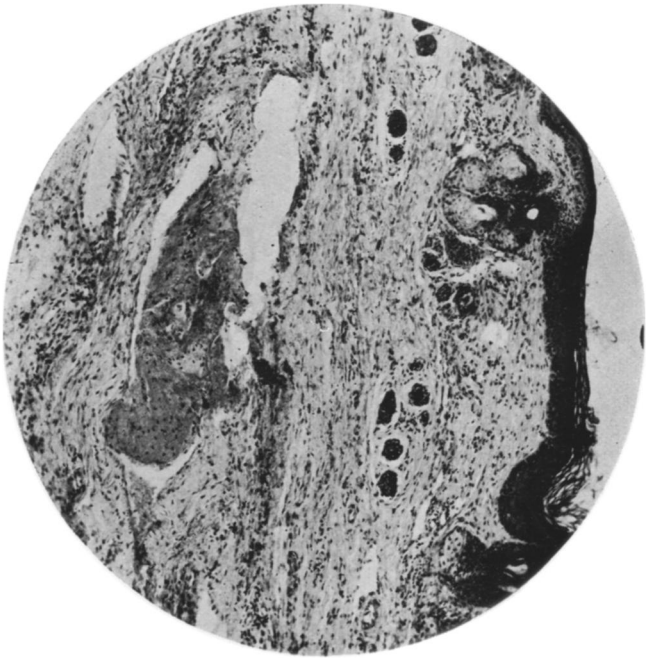

Fig. 13

PLATE 22

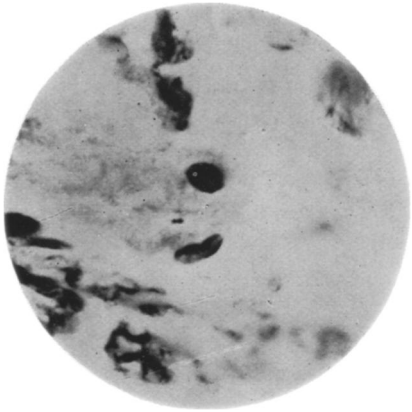

Fig. 14

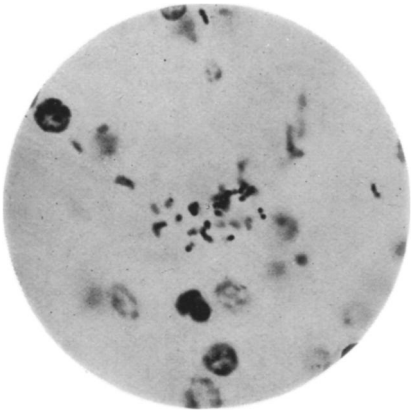

Fig. 15

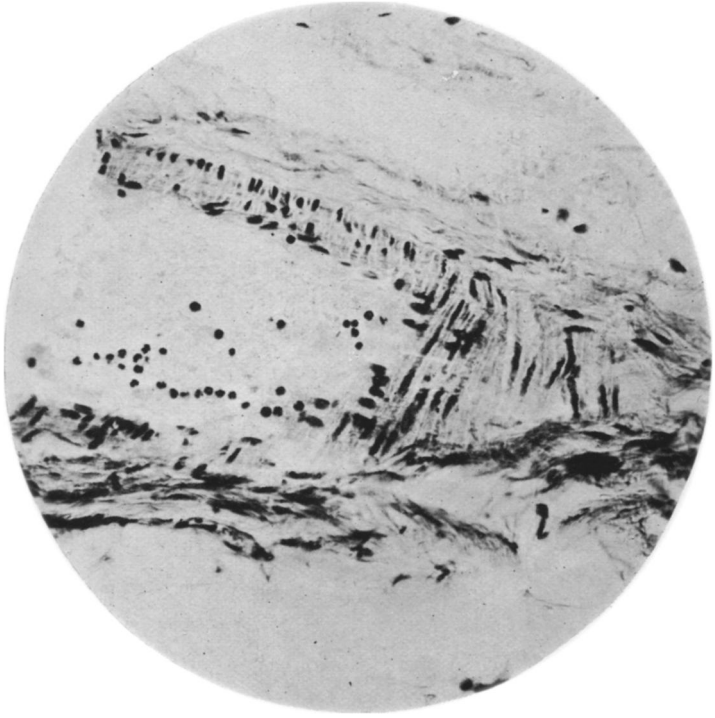

Fig. 16

Fig. 17